

CHARACTERIZATION OF  
A NOVEL DELETION ALLELE  
OF *Brca1*

by

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## **Declaration**

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration, except where specifically indicated in the text. None of the material presented herein has been submitted previously for the purpose of obtaining another degree.

Debrah M. Thompson

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*“Increase the Flash Gordon noise and put more science stuff around.”*

-Crow, MST3K, on how to make things look more “sciency.”

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## ABSTRACT

### Characterization of a novel deletion allele of *Brca1*

*BRCA1* is a breast and ovarian cancer predisposition gene involved in human familial breast cancer. Although its functions are not fully understood, it appears to be involved in DNA damage repair and genome stability. It is only 58% identical to its mouse homologue *Brca1*, but has two highly-conserved domains; an N-terminal zinc-finger RING domain, and two BRCT repeats at the C-terminus.

In this study, two murine knockout alleles of *Brca1* were generated. Both are missing exon 2, which contains the translational start site. The first (*Brca1<sup>Brdm1</sup>*, referred to as "*Brca1<sup>-</sup>*"), has the characteristics of previously described null alleles: *Brca1<sup>+/-</sup>* mice are healthy and not predisposed to tumorigenesis, and neither *Brca1<sup>-/-</sup>* embryonic stem (ES) cells nor mice could be generated. The second allele also replaces exon 2, but this allele (*Brca1<sup>Brdm2</sup>*, named *gollum* and abbreviated as *gol*) does not behave like a null allele: *gol/gol* ES cells are viable and grow normally. The phenotypic differences between these two alleles may be due to the amount of *Brca1* transcript produced by each allele – ~2 kb more genomic sequence from intron 2 is deleted in *gol* than in *Brca1<sup>-</sup>*. This area is postulated to carry a transcriptional repressor. Additionally, the protein produced from the *gol* allele (*Brca1<sup>gol</sup>*) may be more stable than wildtype *Brca1*. *Brca1<sup>gol</sup>* is predicted to lack a significant portion of the highly-conserved N-terminal RING domain, a region known to be important for interactions with protein partners, including Bard1, a nuclear chaperone of *Brca1*. In this study, it was demonstrated that *Brca1<sup>gol</sup>* appears to be able to localize to the nucleus and will form DNA damage-induced nuclear foci, but has a decreased ability to bind to Bard1.

ES cells carrying the *gol* allele were tested for their response to various types of DNA damage. *gol/gol* and *gol/-* ES cells were hypersensitive to  $\gamma$ -irradiation and mitomycin C treatments, which cause double-strand breaks,

but did not appear to be hypersensitive to the base-damaging agents such as ultraviolet light or hydrogen peroxide. This indicated that the cells were deficient in double-strand break repair, which has two main components: homologous recombination repair (HRR) and non-homologous end-joining (NHEJ). Further analysis revealed that *gol/gol* cells had both a slight decrease in HRR efficiency and an increase in NHEJ efficiency as assayed by gene targeting and random plasmid integration.

*gol*, a novel deletion allele of *Brca1*, is of interest not only because it ablates a highly-conserved domain of the protein without conferring the expected loss of viability, but also because it has a clear defect in DNA damage repair. It offers a unique opportunity to further study the functions of *Brca1*.

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## ABBREVIATIONS

5' RACE	Rapid Amplification of cDNA Ends (5' version)
6TG	6-thioguanine
aa	amino acid
AAP	Abridged Anchor Primer
Ab	antibody
AEBSF	4-(2-aminoethyl)benzenesulfonyl fluoride
AR	androgen receptor
AT	ataxia-telangiectasia
ATM	Ataxia telangiectasia mutated
ATR	ATM and RAD3-related
AUAP	Abridged Universal Amplification Primer
BAC	bacterial artificial chromosome
BACH	BRCA1-associated C-terminal helicase
BAP1	BRCA1-associated protein 1 (human protein)
BARD1	BRCA1- associated RING domain partner 1 (human protein)
Bard1	Brca1- associated RING domain partner 1 (mouse protein)
<i>BARD1</i>	BRCA1- associated RING domain partner 1 (human gene)
<i>Bard1</i>	Brca1- associated RING domain partner 1 (mouse gene)
BASC	BRCA1-associated genome surveillance complex
<i>BAX</i>	<i>BCL-associated X</i> , human gene
BCIP/NBT	5-Bromo-4-Chloro-3-Indolyl phosphate/nitro blue tetrazolium
BER	base excision repair
<i>BLM</i>	Bloom's syndrome, causative gene (human)
<i>Blm</i>	Bloom's syndrome, causative gene (mouse)
bp	base pair
<i>BRC-1</i>	<i>C. elegans BRCA1</i> homologue (gene)
<i>BRCA1</i>	<i>Breast cancer 1</i> (human gene)
BRCA1	Breast cancer 1 (human protein)
Brca1	Breast cancer 1 (mouse protein)
<i>Brca1</i>	<i>Breast cancer 1</i> (mouse gene)
<i>Brca1<sup>-</sup></i> (or “-“)	<i>Brca1<sup>Brdm1</sup></i> , null allele with <i>Hprt</i> cassette
<i>Brca1</i> -addPGK-TV	Targeting vector which adds PGK to the <i>c1</i> ( <i>Puro corrected</i> ) allele
<i>Brca1</i> -cond1-TV	<i>Brca1</i> conditional targeting vector, generates <i>Brca1<sup>Brdc1</sup></i>
<i>Brca1</i> -cond2-TV	<i>Brca1</i> conditional targeting vector, generates <i>Brca1<sup>Brdc2</sup></i>
<i>Brca1</i> -fixPuro-TV	<i>Brca1</i> targeting vector which fixes the <i>Puro</i> cassette of <i>c1</i>
Brca1 <sup>gol</sup>	Brca1 protein coded by the <i>Brca1<sup>Brdm2</sup></i> or <i>gol</i> allele

<i>Brca1</i> -gollum-TV	<i>Brca1</i> targeting vector, generates <i>Brca1</i> <sup>Brdm2</sup> or <i>gollum</i>
<i>Brca1</i> -Hprt-TV	<i>Brca1</i> <i>Hprt</i> replacement vector, generates <i>Brca1</i> <sup>Brdm1</sup>
<i>Brca1</i> -Neo-TV	<i>Brca1</i> <i>Neo</i> replacement vector
<i>BRCA2</i>	<i>Breast cancer 2</i> (human gene)
<i>Brca2</i>	<i>Breast cancer 2</i> (mouse gene)
BRCT	BRCA1 C-terminal
<i>BRD-1</i>	<i>C. elegans</i> <i>BARD1</i> homologue
BSA	bovine serum albumin
C	cysteine
<i>c1</i>	<i>Brca1</i> <sup>Brdc1</sup> conditional allele
<i>c1(+neo)</i>	<i>Brca1</i> <sup>Brdc1</sup> conditional allele with <i>Neo</i> selection cassette
<i>c2</i>	<i>Brca1</i> <sup>Brdc2</sup> conditional allele
cM	centiMorgans
CMV	cytomegalovirus
<i>co</i>	conditional allele (in general)
Cre	cyclization recombination
CS	Cockayne's Syndrome
CstF	Cleavage stimulation factor
CtIP	CtBP-interacting protein
CtBP	C-terminal binding protein
Da	Dalton
DIG	digoxigenin
DMEM	Dulbecco's Modified Eagle Medium
DNA-PKcs	DNA-protein kinase, catalytic subunit
DSB	double-strand break
DSBR	double-strand break repair
dsDNA	double-stranded DNA
E	embryonic day
E2F1	E2F-transcription factor 1
ECL	enhanced chemiluminescence
EDTA	ethylenediaminetetraacetic acid
ER	oestrogen receptor
ES	embryonic stem
FANCD2	Fanconi anaemia protein D2 (human)
FBS	foetal bovine serum
FIAU	1-(2'-deoxy-2'-fluoro-b-D-arabinofuranosyl)-5-iodouracil
FISH	Fluorescent <i>in situ</i> hybridization
FITC	fluorescein isothiocyanate
G418	geneticin

<i>GADD45</i>	<i>Growth arrest and DNA damage inducible gene 45</i> (human)
<i>Gapd</i>	<i>Glyceraldehyde-3-phosphate dehydrogenase</i> (mouse gene)
<i>Gdf-9</i>	<i>Growth and differentiation factor-9</i> (mouse gene)
GGR	global genomic repair
<i>gol</i>	<i>Brca1<sup>Brdm2</sup></i> , or <i>gollum</i>
GSP	gene-specific primer
GST	glutathione-S-transferase
H	histidine
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
HAT	hypoxanthine/aminopterin/thymine
HCC1937	human tumour- derived cell line carrying mutated <i>BRCA1</i> and <i>p53</i> genes (among other mutations)
<i>Hprt</i>	<i>hypoxanthine phosphoribosyltransferase</i> (mouse gene)
HRR	homologous recombinational repair
HSV- <i>tk</i>	herpes-simplex virus type 1 <i>thymidine kinase</i> (gene)
HT	hypoxanthine/thymidine
HU	hydroxyurea
i	inosine
IP	immunoprecipitation
JAK	Janus kinase
JNK/SAPK	c-Jun N-terminal kinase/stress-activated protein kinase
kb	kilobase
kDa	kilodalton
LIF	leukocyte inhibitory factor
LOH	loss of heterozygosity
<i>loxP</i>	<i>locus of crossover (P1)</i>
LTR	long terminal repeat
M-10	cell culture medium containing 10% serum
M-15	cell culture medium containing 15% serum
M17S2 ( <i>NBR1</i> )	<i>Membrane component, Chromosome 17, Surface marker 2</i>
<i>MDM2</i>	<i>Mouse Double-Minute homologue 2</i> , human gene
Mdm2	Mouse double-minute 2 (protein)
MEF	mouse embryonic fibroblast
<i>Melk</i>	<i>Maternal embryonic leucine zipper kinase</i> (mouse gene)
MMC	mitomycin C
MMS	methyl methanesulfonate
MMTV	mouse mammary tumour virus
MOPS	3-(N-morpholino)propanesulfonic acid
<i>Nbr1</i>	<i>Neighbour of Brca1 1</i> (mouse gene)
<i>NBR1</i>	<i>Neighbour of BRCA1 1</i> (human gene)

<i>NBR2</i>	<i>Neighbour of BRCA1 2</i> (human gene)
NBS1	Nijmegen breakage syndrome protein
<i>Neo</i>	<i>Neomycin phosphotransferase</i> ; antibiotic-resistance gene
NER	nucleotide excision repair
NES	nuclear export signal
NHEJ	non-homologous end joining
NLS	nuclear localization sequence
<i>p21</i>	<i>p21<sup>Waf1/Cip1</sup></i>
PBS	phosphate-buffered saline
PCNA	proliferating cell nuclear antigen
PGK	promoter from the mouse <i>Phosphoglyceride kinase</i> gene
PI3K	phosphatidylinositol 3-kinase
PR	progesterone receptor
puro	puromycin, antibiotic
<i>Puro</i>	<i>puromycin N-acetyltransferase</i> ; antibiotic-resistance gene
PVDF	polyvinylidene fluoride
pVHL	von Hippel-Lindau protein
RB1	Retinoblastoma protein
<i>RB1</i>	Retinoblastoma gene
revPGK	reversed PGK promoter
RING	Really Interesting New Gene
RIPA	radioimmunoprecipitation
RNA Pol II	RNA Polymerase II holoenzyme
RNAi	RNA interference
RT-PCR	reverse transcription-polymerase chain reaction
scid	severe combined immunodeficiency
SDS	sodium dodecyl sulphate
SET	wash buffer, 0.15M NaCl, 20 mM Tris pH7.8, 1mM EDTA
SKY	spectral karyotyping
ssDNA	single-stranded DNA
STAT	signal transducer and activator of transcription
SV40	simian virus 40
T antigen	tumour antigen
TBST	wash buffer for protein blots (Westerns)
TCR	transcription coupled repair
Tris	Tris-Cl, buffer
TSG	tumour suppressor gene
TV	targeting vector
UTR	untranslated region
UV	ultraviolet

V(D)J recombination	variable (diverse) joining recombination
WT	wildtype
xBARD1	<i>Xenopus</i> homologue of BARD1 (protein)
<i>xBRCA1</i>	<i>Xenopus</i> homologue of <i>BRCA1</i> (gene)
X-gal	5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside
XP	Xeroderma Pigmentosum
YAC	yeast artificial chromosome
Zn	zinc
$\beta$ -gal	$\beta$ -galactosidase
$\beta$ -geo	$\beta$ -galactosidase/Neomycin phosphotransferase fusion gene
$\beta$ -ME	$\beta$ -mercaptoethanol
$\gamma$	gamma
$\Delta$ X.11	<i>Brca1</i> splice isoform lacking exon 11
$\Delta$ X.2	<i>Brca1</i> splice isoform lacking exon 2